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APPLICATION OF MAGNETIC NANOPARTICLES, FLUORESCENT NANOPARTICLES AND NANOZYMES IN IMMUNOASSAYS*

Keywords: carbon coating, iron nanoparticles, europium, chelate, lanthanide, Prussian blue, horseradish peroxidase.

Magnetic carbon-coated-iron nanoparticles (MNP) were used to develop diagnostic reagents for nuclear-magnetic-resonance-based immunoassays. Clusters of MNP were coated with four different proteins: casein, albumin, gelatins A and B and covalently conjugated with recognition molecules (monoclonal antibodies, streptavidin, protein G etc., fig.1A). Ability of MNP to decrease transverse relaxation time of protons (T2) allows their quantitation with the aid of portable relaxometer (Fig. 1B). One can determine concentration of analyte of interest by measuring T2.

Synthesis of MNP-based nanoconjugates was optimized, size of nanoclusters can be tuned via change of synthesis conditions. Long-term stability of nanoclusters was confirmed; their physical-chemical properties were studied [1].

Fluorescent nanoparticles were prepared by precipitation technique [2] from (Z)-2-hydroxy-4-oxo-4-p-tolyl-2-butenoic acid (HOTBA), europium ions and bovine serum albumin (Fig. 1C). Nanoparticles were covalently conjugated with treptococcal protein G. Long-lived luminescence of nanoparticles (λ_{ex} = 350 nm, λ_{em} = 620 nm, time delay = 100 ms) was used to develop time-resolved fluorescent immunoassay of IgG.

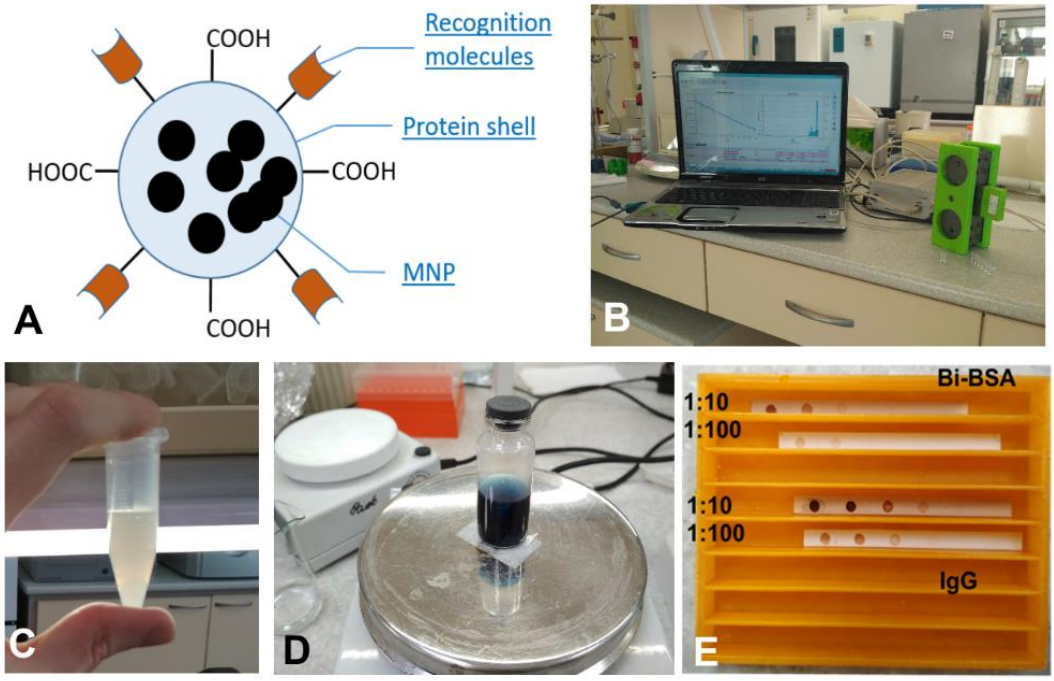

Figure 1. A – structure of protein-coated MNP nanoclusters; B – portable NMR-relaxometer connected to laptop; C – PBN-assisted dot-immunoassay of Bi-BSA and IgG (diaminobenzidine staining); D – preparation of PNB; E – suspension of HOTBA/Eu^{3+}/BSA nanoparticles in water

Prussian blue nanoparticles (PBN) are effective analogs of horseradish peroxidase (nanozymes) [3]. Our purpose is to use PBN as labels in colorimetric immunoassays. We performed preliminary study in which PBN with the size of 100–110 nm were synthesized (Fig. 1D), coated with protein layer and conjugated with streptavidin. Model colorimetric assay of biotinylated albumin and IgG on the nitrocellulose membrane (Fig. 1E).

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